



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

October 22, 2014

MEMORANDUM

Subject: Efficacy Review for BRACE; EPA Reg. No. 777-99; DP Barcode: 420824.

From: Ibrahim Laniyan, Ph.D.
Microbiologist
Product Science Branch
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MOP

Thru: Mark Perry
Acting Team Leader
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To: Thomas Luminello *rec'd* NOV 10 2014
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Reckitt Benckiser Inc.
Morris Corporate Center IV
399 Interpace Parkway
Parsippany, NJ 07054-0225

Formulation from the Label:

<u>Active Ingredients</u>	<u>% by wt.</u>
Ethanol	58.00 %
Alkyl (50% C ₁₄ , 40% C ₁₂ , 10% C ₁₆)	
Dimethyl benzyl ammonium saccharinate.....	0.10 %
Other Ingredients.....	41.90 %
Total.....	100.00 %

I. BACKGROUND

The product, BRACE is a ready-to-use disinfectant (bactericide, tuberculocide, fungicide and virucide) for use on hard, non-porous, non-food contact surfaces in household, institutional, and commercial environments. It is also a sanitizer on hard, non-porous, non-food contact surfaces and on soft (fabric) surfaces. The registrant proposes to amend the Master Label for Brace (EPA Reg. Number 777-99) by adding the organisms and contact times supported by the submitted studies. The registrant is also requesting that the Agency reconsiders the previous unacceptable classification of data generated on *Neisseria elongata* (ATCC 25295), MRID 492486-19. In addition, the registrant proposes to add language to the product master label to include claims that the product is effective against organisms similar to the Middle East Respiratory Syndrome (MERS) Virus. All efficacy studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package identified as D420824 contained a letter from the applicant representative to EPA (dated June 2, 2014), EPA Form 8570-35 (Data Matrix), twenty seven efficacy studies (MRIDs nos. 493877-01 thru -27), Statement of No Data Confidentiality Claims for all studies embedded in each respective MRID, and the proposed label.

II. USE DIRECTIONS

The product is designed to be used for disinfecting hard, non-porous surfaces such as door handles, clean-up carts, light switches, sinks, tubs, tiles, toilets, shower doors, floors, dressing or linen carts, hampers, diaper pails, toilet seats, bed pans, plastic mattress covers, lockers... Directions on the proposed label provided the following information regarding use of the product as a disinfectant:

(Pre-clean surfaces prior to use) Hold can (container) upright 6" to 8" from surface. Spray 3 to 4 seconds until covered with mist. ((Gross) (Heavy) soil must be removed prior to application).

To Sanitize: Let stand for (10) (30) seconds then allow to air dry.

To Disinfect: (Let stand for 3 minutes then allow to air dry). (For *Mycobacterium bovis* BCG Let stand for 6 minutes then allow to air dry). (For (Norovirus) (and) (*Mycobacterium bovis* BCG (Quant tuberculosis)). Let stand for 10 minutes then allow to air dry).

Rinse toys and food contact surfaces with potable water after use.

For surfaces that come in contact with food: Use only on hard, non-porous surfaces and rinse thoroughly with water.

To Disinfect Toys: Use only on hard, non-porous surfaces and rinse thoroughly with water after use. Let stand for 3 minutes then allow to air dry. (For *Mycobacterium bovis* BCG Let stand for 6 minutes then allow to air dry). (For (Norovirus) (and) (*Mycobacterium bovis* BCG (Quant tuberculosis)). Let stand for 10 minutes then allow to air dry).

Rinse child/baby plastic toys, child/baby hard non-porous surfaces and all food contact surfaces with potable water or a damp cloth after use.

To Control and Prevent (the Growth of) Mold & Mildew (and their Odors) (on Hard, Non-porous Surfaces): Apply to pre-cleaned surface. Allow to remain wet for 3 minutes. Let air dry. Repeat applications in weekly intervals or when mold and mildew growth appears.

Fabric Sanitizer: (For spot treatment (2" x 2" area) only).

To (Spot) Sanitize Soft Surfaces (Fabrics): Spray until fabric is wet. DO NOT SATURATE. Fabric must remain wet for 30 seconds. Let air dry. For difficult odors, repeat application.

To (Control) (Eliminate) odor-causing Bacteria on Soft Surfaces (Fabrics): Spray until fabric is wet. DO NOT SATURATE. Let air dry. For difficult odors, repeat application. Reapply as necessary.

For Use in Air: Hold can upright. Spray towards the center of the room.

<< USE DIRECTIONS FOR WIDE SPRAY CAN >>

DIRECTIONS FOR USE: It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Read the entire label before using the product.

(Pre-clean surfaces prior to use.) Hold can (container) upright 6" to 8" from surface. Spray (for) (X) (seconds) until (covered with)(thoroughly) (wet) (mist). ((Gross) (Heavy) soil must be removed prior to application).

To Sanitize: Let stand for (10) 30 seconds then allow to air dry.

To Disinfect: (Let stand for 3 minutes then allow to air dry.) (For Mycobacterium bovis BCG Let stand for 6 minutes then allow to air dry). (For (Norovirus) (and) (Mycobacterium bovis BCG (Quant tuberculosis)). let stand for 10 minutes then allow to air dry.

Rinse toys and food contact surfaces with potable water after use.

For surfaces that come in contact with food: Use only on hard, non-porous surfaces and rinse thoroughly with water.

To Disinfect Toys: Use only on hard, non-porous surfaces and rinse thoroughly with water after use. Let stand for 3 minutes then allow to air dry. (For Mycobacterium bovis BCG Let stand for 6 minutes then allow to air dry). (For (Norovirus) (and) (Mycobacterium bovis BCG (Quant tuberculosis)), let stand for 10 minutes then allow to air dry).

Rinse child/baby plastic toys, child/baby hard non-porous surfaces and all food contact surfaces with potable water or a damp cloth after use.

To Control and Prevent (the Growth of) Mold & Mildew (and their Odors) (on Hard, Non-porous Surfaces): Apply to pre-cleaned surface. Allow to remain wet for 3 minutes. Let air dry. Repeat applications in weekly intervals or when mold and mildew growth appears.

Fabric Sanitizer: (For spot treatment (2" x 2" area) only).

To (Spot) Sanitize Soft Surfaces (Fabrics): Spray until fabric is wet. DO NOT SATURATE. Fabric must remain wet for 30 seconds. Let air dry. (For difficult odors, repeat application).

To (Control) (Eliminate) odor-causing Bacteria on Soft Surfaces (Fabrics): Spray until fabric is wet. DO NOT SATURATE. Let air dry. (For difficult odors, repeat application). Reapply as necessary.

For Use in Air: Hold can upright. Spray towards the center of the room.

<< USE DIRECTIONS FOR TB Claim without soil >>

DIRECTIONS FOR USE: It is a violation of Federal law to use this product in a manner inconsistent with its labeling. Read the entire label before using the product.

Pre-clean surfaces prior to use. Hold can (container) upright 6" to 8" from surface. ((Gross) (Heavy) soil must be removed prior to application).

To Disinfect: (Let stand for (2) (3) (5) minutes then allow to air dry).

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots at the LCL. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products) may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of 10^4 - 10^5 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots at LCL must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots at the LCL of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate

complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Sanitizer Test (for inanimate, non-food contact surfaces): The effectiveness of sanitizers for non-food contact surfaces must be supported by data that show that the product will substantially reduce the numbers of test bacteria on a treated surface over those on an untreated control surface. The test surface(s) should represent the type(s) of surfaces recommended for treatment on the label, i.e., porous or non-porous. Products that are represented as "one-step sanitizers" should be tested with an appropriate organic soil load, such as 5 percent serum. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Staphylococcus aureus* (ATCC 6538) and either *Klebsiella pneumoniae* (aberrant, ATCC 4352) or *Enterobacter aerogenes* (ATCC 13048 or 15038). Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV. BRIEF DESCRIPTION OF THE DATA

Note: The product lots 2001-106, 2001-112, 2028-029 and 2028-030, were tested at the respective concentrations of 0.081% Onyoxide/55.96% Ethanol, 0.081% Onyoxide/54.67% Ethanol, 0.084% Onyoxide/54.16% Ethanol and 0.084% Onyoxide/54.516% Ethanol.

1. MRID 493877-01, "AOAC Tuberculocidal Activity of Disinfectant Spray Products,"
Test Organism: *Mycobacterium bovis* - BCG. For product Formula #e0029-002I,
Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Jill Ruhme,
B.S. Study completion date – February 3, 2014. Project Number A15530.

This study was conducted against *Mycobacterium bovis*-BCG. Testing was conducted using two batches of test substance; Formula #e0029-002I (Batch 2001-106 and Batch 2001-112) is was tested using ATS Laboratory Protocol No. SRC62080913.TB.1 (copy provided). The product was received as ready to use (RTU) aerosol spray. A ≤ 10 μ L loop of stock slant culture was transferred into 20 mL tubes of Modified Proskauer-Beck Broth and incubated for 21 days at 35-37°C, undisturbed, in a slanted position. Following incubation, the test culture was transferred to a sterile tissue grinder containing 1.00 mL of 0.85% saline + 0.1 % tween 80 using a transfer loop. The culture was macerated to break up large clumps or aggregates of the test organism. A 9.0 mL aliquot of Modified Proskauer-Beck broth was added to the culture and the suspension was transferred from the tissue grinder to a sterile test tube. The suspension was allowed to settle for approximately 10-15 minutes. The upper portion of culture was removed, leaving behind any debris or clumps, and transferred to a sterile vessel. This culture was standardized to 20.51% Transmittance (% T) at 650 nm by dilution with Modified Proskauer-Beck broth. A 10 μ L aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 50% relative humidity.

Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (23.82°C) and at 39.7% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to vessels containing the neutralizer and were shaken. The carriers were then transferred to a vessel containing 20 mL of Modified Proskauer-Beck Broth. Within approximately 30 minutes of neutralization, a 2.0 mL aliquot of the neutralizer was transferred to individual vessels containing 20 mL of Middlebrook 7H9 and 20 mL of Kirchner's Medium. All broth subcultures were incubated at 35-37°C and examined for growth following 30 and 61 day incubation periods. All plates were incubated for 19 days at 35-37°C. Following incubation, the subcultures and plates were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments reported in this study were reviewed.

2. MRID 493877-02, "AOAC Tuberculocidal Activity of Disinfectant Spray Products,"
Test Organism: *Mycobacterium bovis* - BCG. For product Formula #e0029-002I,
Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Jill Ruhme,
B.S. Study completion date – February 12, 2014. Project Number A15531.

This study was conducted against *Mycobacterium bovis*-BCG. Testing was conducted using two batches of test substance; Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. This was tested using ATS Laboratory Protocol No. SRC62080913.TB.2 (copy provided). The product was received as ready to use (RTU) aerosol spray. A ≤ 10 μ L loop of stock slant culture was transferred into 20 mL tubes of Modified Proskauer-Beck Broth and incubated for 21 days at 35-37°C, undisturbed, in a slanted position. Following incubation, the test culture was transferred to a sterile tissue grinder containing 1.00 mL of 0.85% saline + 0.1 % tween 80 using a transfer loop. The culture was macerated to break up large clumps or aggregates of the test organism. A 9.0 mL aliquot of Modified Proskauer-Beck broth was added to the culture and the suspension was transferred from the tissue grinder to a sterile test tube. The suspension was allowed to settle for approximately 10-15 minutes. The upper portion of culture was removed, leaving behind any debris or clumps, and transferred to a sterile vessel. This culture was standardized to 20.51% Transmittance (% T) at 650 nm by dilution with Modified Proskauer-Beck broth. A 10 μ L aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 50% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 3 minutes at room temperature (23.38°C) and at 40.23% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to vessels containing the neutralizer and were shaken. The carriers were then transferred to a vessel containing 20 mL of Modified Proskauer-Beck Broth. Within approximately 30 minutes of neutralization, a 2.0 mL aliquot of the neutralizer was transferred to individual vessels containing 20 mL of Middlebrook 7H9 and 20 mL of Kirchner's Medium. All broth subcultures were incubated at 35-37°C and examined for growth following 30 and 61 day incubation periods. All plates were incubated for 19 days at 35-37°C. Following incubation, the subcultures and plates were

examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments reported in this study were reviewed.

3. MRID 493877-03, "AOAC Tuberculocidal Activity of Disinfectant Spray Products,"
Test Organism: *Mycobacterium bovis* - BCG. For product Formula #e0029-002I,
Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Jill Ruhme,
B.S. Study completion date – February 12, 2014. Project Number A15532.

This study was conducted against *Mycobacterium bovis*-BCG. Testing was conducted using two batches of test substance; Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. This was tested using ATS Laboratory Protocol No. SRC62080913.TB.3 (copy provided). The product was received as ready to use (RTU) aerosol spray. A ≤ 10 μ L loop of stock slant culture was transferred into 20 mL tubes of Modified Proskauer-Beck Broth and incubated for 21 days at 35-37°C, undisturbed, in a slanted position. Following incubation, the test culture was transferred to a sterile tissue grinder containing 1.00 mL of 0.85% saline + 0.1 % tween 80 using a transfer loop. The culture was macerated to break up large clumps or aggregates of the test organism. A 9.0 mL aliquot of Modified Proskauer-Beck broth was added to the culture and the suspension was transferred from the tissue grinder to a sterile test tube. The suspension was allowed to settle for approximately 10-15 minutes. The upper portion of culture was removed, leaving behind any debris or clumps, and transferred to a sterile vessel. This culture was standardized to 20.51% Transmittance (% T) at 650 nm by dilution with Modified Proskauer-Beck broth. A 10 μ L aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 50% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 5 minutes at room temperature (24.35°C) and at 39.10% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to vessels containing the neutralizer and were shaken. The carriers were then transferred to a vessel containing 20 mL of Modified Proskauer-Beck Broth. Within approximately 30 minutes of neutralization, a 2.0 mL aliquot of the neutralizer was transferred to individual vessels containing 20 mL of Middlebrook 7H9 and 20 mL of Kirchner's Medium. All broth subcultures were incubated at 35-37°C and examined for growth following 30 and 61 day incubation periods. All plates were incubated for 19 days at 35-37°C. Following incubation, the subcultures and plates were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments reported in this study were reviewed.

4. MRID 493877-04, "AOAC Tuberculocidal Activity of Disinfectant Spray Products,"
Test Organism: *Mycobacterium bovis* - BCG. For product Formula #e0029-002I,
Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Jill Ruhme,
B.S. Study completion date – February 12, 2014. Project Number A15533.

This study was conducted against *Mycobacterium bovis*-BCG. Testing was conducted using two batches of test substance; Formula #e0029-002I, Batch 2001-106 and Batch 2001-112.

This was tested using ATS Laboratory Protocol No. SRC62080913.TB.5 (copy provided). The product was received as ready to use (RTU) aerosol spray. A ≤ 10 μL loop of stock slant culture was transferred into 20 mL tubes of Modified Proskauer-Beck Broth and incubated for 21 days at 35-37°C, undisturbed, in a slanted position. Following incubation, the test culture was transferred to a sterile tissue grinder containing 1.00 mL of 0.85% saline + 0.1 % tween 80 using a transfer loop. The culture was macerated to break up large clumps or aggregates of the test organism. A 9.0 mL aliquot of Modified Proskauer-Beck broth was added to the culture and the suspension was transferred from the tissue grinder to a sterile test tube. The suspension was allowed to settle for approximately 10-15 minutes. The upper portion of culture was removed, leaving behind any debris or clumps, and transferred to a sterile vessel. This culture was standardized to 20.51% Transmittance (% T) at 650 nm by dilution with Modified Proskauer-Beck broth. A 10 μL aliquot of fetal bovine serum was added to 1.90 mL of the prepared broth culture to yield a 5% fetal bovine serum organic soil load. A 10 μL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 50% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 6 minutes at room temperature (23.85°C) and at 40.98% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at staggered intervals to vessels containing the neutralizer and were shaken. The carriers were then transferred to a vessel containing 20 mL of Modified Proskauer-Beck Broth. Within approximately 30 minutes of neutralization, a 2.0 mL aliquot of the neutralizer was transferred to individual vessels containing 20 mL of Middlebrook 7H9 and 20 mL of Kirchner's Medium. All broth subcultures were incubated at 35-37°C and examined for growth following 30 and 61 day incubation periods. All plates were incubated for 19 days at 35-37°C. Following incubation, the subcultures and plates were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol amendments and deviations reported in this study were reviewed.

5. MRID 493877-05, "AOAC Germicidal Spray Method," Test Organism: *Shigella flexneri* serotype 1B (ATCC 9380). For product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Gracia Schroeder, B.S. Study completion date – March 6, 2014. Project Number A16010.

This study was conducted against *Shigella flexneri* serotype 1B (ATCC 9380). Testing was conducted using two batches of test substance; Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. This was tested using ATS Laboratory Protocol No. SRC62121113.GS.8 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24 \pm 2 hours at 35-37°C. Following incubation a 10 μL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). Two additional daily transfers were prepared. The final test culture was incubated for 48-54 hours at 35-37°C. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. A 10 μL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product

batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C and at 60% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (20.3°C) and at 14.3% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Lethen Broth + 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures and plates were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

6. MRID 493877-06, "AOAC Germicidal Spray Method," Test Organism: *Salmonella enteritidis* (ATCC 4931). For product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Gracia Schroeder, B.S. Study completion date – March 6, 2014. Project Number A16011.

This study was conducted against *Salmonella enteritidis* (ATCC 4931). Testing was conducted using two batches of test substance; Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. This was tested using ATS Laboratory Protocol No. SRC62121113.GS.7 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). Two additional daily transfers were prepared. The final test culture was incubated for 48-54 hours at 35-37°C. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 52.8% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (20.6°C) and at 17.3% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Lethen Broth + 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures and plates were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

7. MRID 493877-07, "AOAC Germicidal Spray Method," Test Organism: *Staphylococcus haemolyticus* (ATCC 29969). For product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Gracia Schroeder, B.S. Study completion date – March 5, 2014. Project Number A16012.

This study was conducted against *Staphylococcus haemolyticus* (ATCC 29969). Testing was conducted using two batches of test substance; Formula #e0029-002I, Batch 2001-106 and

Batch 2001-112. This was tested using ATS Laboratory Protocol No. SRC62121113.GS.10 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). Two additional daily transfers were prepared. The final test culture was incubated for 48-54 hours at 35-37°C. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 53.7% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (20.6°C) and at 14% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures and plates were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

8. MRID 493877-08, "AOAC Germicidal Spray Method," Test Organism: *Staphylococcus hominis* (ATCC 25615). For product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Joshua Luedtke, M.S. Study completion date – February 4, 2014. Project Number A16017.

This study was conducted against *Staphylococcus hominis* (ATCC 25615). Testing was conducted using two batches of test substance; Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. This was tested using ATS Laboratory Protocol No. SRC62121113.GS.11 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). Two additional daily transfers were prepared. The final test culture was incubated for 48-54 hours at 35-37°C. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 40% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (20.6°C) and at 17.7% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures and plates were examined for the presence or absence of

visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

9. MRID 493877-09, "AOAC Germicidal Spray Method," Test Organism: *Staphylococcus saprophyticus* (ATCC 15305). For product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Joshua Luedtke, M.S. Study completion date – February 12, 2014. Project Number A16018.

This study was conducted against *Staphylococcus saprophyticus* (ATCC 15305). Testing was conducted using two batches of test substance; Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. This was tested using ATS Laboratory Protocol No. SRC62121113.GS.12 (copy provided). The product was received as ready to use (RTU) aerosol spray. A loop of stock slant culture was transferred to an initial 10 mL tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). Two additional daily transfers were prepared. The final test culture was incubated for 48-54 hours at 35-37°C. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. A 10 µL aliquot of the prepared culture was uniformly spread over 10 individual glass slide carriers (18 mm x 36 mm) per product batch, each in a Petri dish matted with two pieces of filter paper. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 53.1% relative humidity. Carriers were used within 2 hours of drying. Test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (20.3°C) and at 19.5% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Lethen Broth + 0.1% Sodium Thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures and plates were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

10. MRID 493877-10, "AOAC Germicidal Spray Method," Test Organism: *Micrococcus luteus* (ATCC 14408) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Matthew Sathe, B.S. Study conducted at ATS Labs. Study completion date – May 16, 2014. Project Number A16229.

This study was conducted against *Micrococcus luteus* (ATCC 14408). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62020314.GS (copy provided). The product was received ready to use (RTU) aerosol spray. A loopful of stock was transferred to an initial 10 mL tube of Synthetic Broth growth medium and was incubated 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). An additional transfer was prepared for testing on April 29, 2014. The final test culture was incubated for 48-54 hours at 35-37°C and was thoroughly mixed prior to use. For testing on March 27, 2014, a 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and

covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C and at 65% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (20.5-20.97°C) and at 28.52-30.5% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Lethen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. The entire volume of the subculture broths were individually transferred to the surface of a filter membrane (0.45 µm porosity) pre-wetted with 10.0 mL of sterile saline and filtered using a vacuum pump. Each filter was washed with ≥50 mL of sterile saline. Each filter was aseptically removed from the filter unit and placed on the surface of a BAP plate. All subcultures were incubated for 2-4 days at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Testing performed Feb. 18, 2014, resulted in multiple control failures and invalid data. The testing was repeated on March 27, 2014, and resulted in valid data. Per sponsor request, **Batch 2001-106 testing was repeated on April 29, 2014 without a soil load and resulted in valid data.** All valid data is contained in the body of the report and invalid data in Attachment 1 of the report.

Note: Protocol amendments were reviewed.

11. MRID 493877-11, "AOAC Germicidal Spray Method," Test Organism *Citrobacter freundii* (ATCC 8090) for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – February 25, 2014. Project Number A16025.

This study was conducted against *Citrobacter freundii* (ATCC 8090). Testing was conducted for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62121113.GS.2 (copy provided). The product was received as a ready to use (RTU) trigger spray. A loopful of stock slant culture was transferred to an initial 10 mL tube of nutrient broth growth medium, mixed and incubated for 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C. The final test culture was mixed thoroughly prior to use. A 0.10 ml aliquot of FBS was added to 1.90 ml of prepared culture to yield a 5% fetal bovine serum organic soil load. Individual glass slide carriers were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. The carriers were allowed to dry for 30 minutes at 25-30°C and at a 60% relative. Carriers were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers were sprayed, in an undisturbed horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches for 2 seconds. Following the spray treatment, each treated carrier was held at room temperature (21.9°C) and 16.5% relative humidity for 2 minutes. At the end of the exposure time, the excess liquid was drained off the carrier. Each treated carrier was then transferred using sterile forceps and following identical staggered intervals to 20 ml aliquots of neutralizing subculture medium. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for

purity, organic soil sterility, carrier stability, Neutralizing Subculture Medium Sterility, viability, neutralization confirmation, and carrier population.

12. MRID 493877-12, "AOAC Germicidal Spray Method," Test Organism *Corynebacterium xerosis* (ATCC 7711) for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – April 23, 2014. Project Number A16026.

This study was conducted against *Corynebacterium xerosis* (ATCC 7711). Testing was conducted for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62121113.GS.3 (copy provided). The product was received as a ready to use (RTU) trigger spray. From stock, sufficient agar plates were inoculated with the test organism. The plates were incubated aerobically for 2 days at 35-37°C. Following incubation, the test organism growth was transferred to sterile diluent to match a 2.0 McFarland Turbidity Standard, targeting 1×10^8 CFU/ml. The culture was then macerated with a tissue grinder to ensure homogeneity. The final test culture was mixed thoroughly prior to use. A 0.10 ml aliquot of FBS was added to 1.90 ml of prepared culture to yield a 5% fetal bovine serum organic soil load. Individual glass slide carriers were each inoculated with 10.0 µL of culture using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. The carriers were allowed to dry for 30 minutes at 35-37°C and at a 52.4% relative humidity. Carriers were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers were sprayed, in an undisturbed horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches for 2 seconds. Following the spray treatment, each treated carrier was held at room temperature (21.6°C) and 15.0% relative humidity for 2 minutes. At the end of the exposure time, the excess liquid was drained off the. Each treated carrier was then transferred using sterile forceps and following identical staggered intervals to 20 ml aliquots of primary neutralizing subculture medium. The carriers were transferred into individual secondary subcultures containing 20 ml aliquots of secondary subculture medium within approximately 25-60 minutes of the initial transfer and the vessel was shaken thoroughly. All subcultures were incubated for 3 days at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic soil sterility, carrier stability, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

Note: Protocol amendments and deviations for this experiment were reviewed.

13. MRID 493877-13, "AOAC Germicidal Spray Method," Test Organism *Enterobacter cloacae* (ATCC 13047) for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – February 18, 2014. Project Number A16027.

This study was conducted against *Enterobacter cloacae* (ATCC 13047). Testing was conducted for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62121113.GS.4 (copy provided). The product was received as a ready to use (RTU) trigger spray. A loopful of stock slant culture was transferred to an initial 10 ml tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 25-30°C. Following incubation, a 10 µl aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 ml of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 25-30°C and was pooled in a sterile vessel and

mixed. The culture was diluted using sterile growth medium by combining 1.00 ml of test organism suspension with 4.00 ml of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.10 ml aliquot of FBS was added to 1.90 ml of prepared culture to yield a 5% fetal bovine serum organic soil load. Individual glass slide carriers were each inoculated with 10.0 μ l of culture using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. The carriers were allowed to dry for 30 minutes at 35-37°C and at a 54.8% relative humidity and appeared visibly dry following drying. Carriers were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers were sprayed, in an undisturbed horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches for 2 seconds. Following the spray treatment, each treated carrier was held at room temperature (20.5°C) and 18.8% relative humidity for 2 minutes. At the end of the exposure time, the excess liquid was drained off the carrier. Each treated carrier was then transferred using sterile forceps and following identical staggered intervals to 20 ml aliquots of neutralizing subculture medium. The vessel was shaken thoroughly. All subcultures were incubated for 48 \pm 2 hours at 25-30°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic soil sterility, carrier stability, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

14. MRID 493877-14, "AOAC Germicidal Spray Method," Test Organism *Klebsiella oxytoca* (ATCC 13182) for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – February 18, 2014. Project Number A16028.

This study was conducted against *Klebsiella oxytoca* (ATCC 13182). Testing was conducted for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62121113.GS.5 (copy provided). The product was received as a ready to use (RTU) trigger spray. A loopful of stock slant culture was transferred to an initial 10 ml tube of growth medium. The tube was mixed and the initial culture was incubated for 24 \pm 2 hours at 35-37°C. Following incubation, a 10 μ l aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 ml of culture medium. The final test culture was incubated for 48-54 hours at 35-37°C. The culture was diluted using sterile growth medium by combining 1.50 ml of test organism suspension with 1.50 ml of sterile growth medium. The final test culture was mixed thoroughly prior to use. A 0.10 ml aliquot of FBS was added to 1.90 ml of prepared culture to yield a 5% fetal bovine serum organic soil load. Individual glass slide carriers were each inoculated with 10.0 μ L of culture using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. The carriers were allowed to dry for 30 minutes at 35-37°C and at a 55.6% relative humidity. Carriers were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers were sprayed, in an undisturbed horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches for 2 seconds. Following the spray treatment, each treated carrier was held at room temperature (20.1°C) and 19.1% relative humidity for 2 minutes. At the end of the exposure time, the excess liquid was drained off the carrier. Each treated carrier was then transferred using sterile forceps and following identical staggered intervals to 20 ml aliquots of neutralizing subculture medium. All subcultures were incubated for 48 \pm 2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic soil sterility, carrier stability, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

15. MRID 493877-15, "AOAC Germicidal Spray Method,," Test Organism *Shigella sonnei* (ATCC 25931) for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Gracia Schroeder, B.S. Study completion date – February 17, 2014. Project Number A16029.

This study was conducted against *Shigella sonnei* (ATCC 25931). Testing was conducted for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62121113.GS.9 (copy provided). The product was received as a ready to use (RTU) trigger spray. A loopful of stock slant culture was transferred to an initial 10 ml tube of growth medium. The tube was mixed and the initial culture was incubated for 24±2 hours at 35-37°C. Following incubation, a 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 ml of culture medium. The final test culture was incubated for 48-54 hours at 35-37°C. The final test culture was mixed thoroughly prior to use. A 0.10 ml aliquot of FBS was added to 1.90 ml of prepared culture to yield a 5% fetal bovine serum organic soil load. Individual glass slide carriers were each inoculated with 10.0 µl of culture using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. The carriers were allowed to dry for 30 minutes at 25-30°C and at a 60% relative humidity and appeared visibly dry following drying. Carriers were used in the test procedure within 2 hours of drying. For each lot of test substance, test carriers were sprayed, in an undisturbed horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches for two seconds. Following the spray treatment, each treated carrier was held at room temperature (23.6°C) and 15.1% relative humidity for 2 minutes. At the end of the exposure time, the excess liquid was drained off the carrier and each treated carrier was then transferred using sterile forceps and following identical staggered intervals to 20 ml aliquots of neutralizing subculture medium. The vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic soil sterility, carrier stability, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

16. MRID 493877-16, "AOAC Germicidal Spray Method,," Test Organism *Streptococcus agalactiae* (ATCC 13813) for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. Study conducted at ATS Labs by Matthew Sathe, B.S. Study completion date – February 18, 2014. Project Number A16030.

This study was conducted against *Streptococcus agalactiae* (ATCC 13813). Testing was conducted for product Formula #e0029-0021 Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62121113.GS.13 (copy provided). The product was received as a ready to use (RTU) trigger spray. From a stock culture of the test organism 10 ml tube of growth medium (BHI broth) was inoculated, mixed, and incubated for 24±2 hours at 35-37°C. Following incubation, a 10 µl aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 ml of culture medium. The final test culture was incubated for 48-54 hours at 35-37°C. The final test culture was mixed thoroughly prior to use. A 0.10 ml aliquot of FBS was added to 1.90 ml of prepared culture to yield a 5% fetal bovine serum organic soil load. Individual glass slide carriers were each inoculated with 10.0 µl of culture using a calibrated pipettor. The inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide contained in the Petri dish. The dish was covered immediately and the procedure repeated until all slides were individually inoculated. The carriers were allowed to dry for 30 minutes at 25-30°C and at a 60% relative humidity. Carriers were used in the test procedure within

2 hours of drying. For each lot of test substance, test carriers were sprayed, in an undisturbed horizontal position, at staggered intervals with the test substance at a distance of 6-8 inches for 2 seconds. Following the spray treatment, each treated carrier was held at room temperature (23°C) and 16.13% relative humidity for 2 minutes. At the end of the exposure time, the excess liquid was drained off the carrier and each treated carrier was then transferred using sterile forceps and following identical staggered intervals to 20 ml aliquots of primary neutralizing subculture medium. The carriers were transferred into individual secondary subcultures containing 20 ml aliquots of secondary subculture medium within approximately 25-60 minutes of the initial transfer and the vessel was shaken thoroughly. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic soil sterility, carrier stability, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

17. MRID 493877-17, "AOAC Germicidal Spray Method," Test Organism: *Vibrio cholerae* (ATCC 11623) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Matthew Sathe, B.S. Study conducted at ATS Labs. Study completion date – April 23, 2014. Project Number A16031.

This study was conducted against *Vibrio cholerae* (ATCC 11623). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62121113.GS.14 (copy provided). The product was received ready to use (RTU) aerosol spray. From stock, sufficient Tryptic Soy Agar with 5% Sheep Blood (BAP) agar plates were inoculated with the test organism and were incubated for 2 days at 35-37°C. Following incubation, the test organism was suspended in Fluid Thioglycollate Medium. The final test culture was thoroughly mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C and at 70% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (22.0°C) and at 16.8% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Lethen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. All subcultures were incubated for 3 days at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

18. MRID 493877-18, "AOAC Germicidal Spray Method," Test Organism: *Bordetella avium* (ATCC 35086) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Matthew Sathe, B.S. Study conducted at ATS Labs. Study completion date – April 23, 2014. Project Number A16288.

This study was conducted against *Bordetella avium* (ATCC 35086). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62013014.GS (copy provided). The product was received ready to use (RTU) aerosol spray. A loopful of stock was transferred to an initial 10 mL

tube of Nutrient Broth growth medium and was incubated 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was thoroughly mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C and at 66% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 3 seconds. The carriers were allowed to remain wet for 3 minutes at room temperature (21.2°C) and at 10.1% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. All subcultures were incubated for 2 days at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

19. MRID 493877-19, "AOAC Germicidal Spray Method," Test Organism: *Escherichia coli* O104:H21 (ATCC BAA-182) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Kristen Niehaus, B.A. Study conducted at ATS Labs. Study completion date – May 15, 2014. Project Number A16647.

This study was conducted against *Escherichia coli* O104:H21 (ATCC BAA-182). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62050214.GS.1 (copy provided). The product was received ready to use (RTU) aerosol spray. A loopful of stock was transferred to an initial 10 mL tube of Synthetic Broth growth medium and was incubated 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1) with one additional daily transfer. The final test culture was incubated for 48-54 hours at 35-37°C and was thoroughly mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C (36.0-36.1°C) and at 55.8% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (23.4°C) and at 34.1% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

20. MRID 493877-20, "AOAC Germicidal Spray Method," Test Organism:

***Escherichia coli* 045:K-H- (ECL1001) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Kristen Niehaus, B.A. Study conducted at ATS Labs. Study completion date – May 15, 2014. Project Number A16648.**

This study was conducted against *Escherichia coli* 045:K-H- (ECL1001). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62050214.GS.2 (copy provided). The product was received ready to use (RTU) aerosol spray. A loopful of stock was transferred to an initial 10 mL tube of Synthetic Broth growth medium and was incubated 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was thoroughly mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 31 minutes at 35-37°C (35.9-36.0°C) and at 55.2% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (22.4°C) and at 37.7% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Lethen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

21. MRID 493877-21, "AOAC Germicidal Spray Method," Test Organism: *Escherichia coli* 0111:H8 (ATCC BAA-184) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Kristen Niehaus, B.A. Study conducted at ATS Labs. Study completion date – May 15, 2014. Project Number A16649.

This study was conducted against *Escherichia coli* 0111:H8 (ATCC BAA-184). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62050214.GS.3 (copy provided). The product was received ready to use (RTU) aerosol spray. A loopful of stock was transferred to an initial 10 mL tube of Synthetic Broth growth medium and was incubated 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was thoroughly mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C (36.0°C) and at 41% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room

temperature (21.6°C) and at 45.2% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Lethen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Subcultures were stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

22. MRID 493877-22, "AOAC Germicidal Spray Method," Test Organism: *Yersinia enterocolitica* (ATCC 23715) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Joshua Luedtke, M.S. Study conducted at ATS Labs. Study completion date – Feb. 4, 2014. Project Number A16032.

This study was conducted against *Yersinia enterocolitica* (ATCC 23715). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62121113.GS.15 (copy provided). The product was received ready to use (RTU) aerosol spray. From stock, sufficient Tryptic Soy Agar with 5% Sheep's Blood (BAP) agar plates were inoculated with the test organism. The plates were incubated for two days at 35-37°C. Following incubation, the organism was suspended in sterile diluent to match a 1.0 McFarland turbidity standard. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C and at 55.8% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (23.6°C) and at 16.6% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Lethen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

23. MRID 493877-23, "AOAC Germicidal Spray Method," Test Organism: *Acinetobacter haemolyticus* (ATCC 17906) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Joshua Luedtke, M.S. Study conducted at ATS Labs. Study completion date – May 7, 2014. Project Number A16529.

This study was conducted against *Acinetobacter haemolyticus* (ATCC 17906). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62032414.GS.1 (copy provided). The product was received ready to use (RTU) aerosol spray. A loopful of stock was transferred to an initial 10 mL tube of Nutrient Broth growth medium and was incubated 24±2 hours at 35-37°C. A 10 µL aliquot of culture was transferred to a 20 x 150 mm Morton Closure tube containing 10 mL of culture medium (daily transfer #1). The final test culture was incubated for 48-54 hours at 35-37°C and was thoroughly mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide

carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 35-37°C (36.1-36.2°C) and at 51.5% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (23.43°C) and at 29.70% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

24. RID 493877-24, "AOAC Germicidal Spray Method," Test Organism: *Pasteurella multocida* (ATCC 12947) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Joshua Luedtke, M.S. Study conducted at ATS Labs. Study completion date – May 7, 2014. Project Number A16530.

This study was conducted against *Pasteurella multocida* (ATCC 12947). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62032414.GS.2 (copy provided). The product was received ready to use (RTU) aerosol spray. From stock, sufficient Tryptic Soy Agar with 5% Sheep's Blood (BAP) agar plates were inoculated with the test organism. The plates were incubated for two days at 35-37°C. Following incubation, the organism was suspended in sterile diluent to appear heavier than a 4.0 McFarland turbidity standard. The final test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C (27.0-27.3°C) and at 70% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (21.76°C) and at 35.51% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of Letheen Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. All subcultures were incubated for 2 days at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

25. MRID 493877-25, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces," Virus: Avian Influenza A (H7N9) Virus, CDC # 2013759189, Strain wildtype A/Anhui/1 /2013, for product Formula #1178-172, Batch # 2028-029 and Batch # 2028-030, by Shanen Conway, B.S. Study conducted at ATS Labs. Study completion date – March 14, 2014. Project Number A16121.

This study was conducted against: Avian Influenza A (H7N9) Virus, CDC # 013759189,

Strain wildtype A/Anhui/1 /2013. Testing was conducted for product Formula #1178-172, Batch # 2028-029 and Batch # 2028-030. These were tested using ATS Laboratory Protocol No. SRC62010914.AFLU (copy provided). The product was received ready to use (RTU) aerosol spray. The test medium used in this study was DMEM supplemented with 10 µg/ml gentamicin, 100 units/ml penicillin, 2.5 µg/ml amphotericin B, 0.2% BSA fraction V, 25 mM HEPES, and 2 µg/ml TPCK-Trypsin. The stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for ten minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at -70°C until the day of use. On the day of use, an aliquot of stock virus was removed, thawed and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 200 µL of virus uniformly over the bottoms of three separate 100 x 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus films were dried at 20.0°C of 4.8% relative humidity until visibly dry (20 minutes). For each lot of test substance, one dried virus film was sprayed with the test substance from a distance of 6-8 inches for 2 seconds until thoroughly. The carriers were exposed for 2 minutes at 20±2°C (20.0°C). Just prior to the end of the exposure time, the plates were individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures were immediately passed through individual Sephadex columns utilizing the syringe plungers in order to detoxify the mixtures. The filtrates (10:1 dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity.

26. MRID 493877-26, "Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces (Modification for Spray Product Application)," Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048), for product Formula #e0029-002I, Lot 2001-106 and Lot 2001-112, by Joshua Luedtke, M.S. Study conducted at ATS Labs. Study completion date – April 22, 2014. Project Number A16077.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Testing was conducted for product Formula #e0029-002I, Lot 2001-106 and Lot 2001-112. These were tested using ATS Laboratory Protocol No. SRC62122113.NFS (copy provided). The product was received ready to use (RTU) aerosol spray. Broth cultures of the test organisms were prepared by inoculation of an initial tube (10 mL) of culture broth from a stock slant ≤30 days old (initial broth suspension). From this, 3 daily transfers using 1 loopful (10 µL) of culture into 10 mL of culture medium, using Nutrient Broth for *Staphylococcus aureus* incubated at 35-37°C and Tryptic Soy Broth for *Enterobacter aerogenes* incubated at 25-30°C. The final test cultures were incubated for 24±2 hours and were thoroughly mixed prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. Sterile 1"x1" glass carriers were inoculated with 0.02 ml (20.0 µL) of culture using a calibrated pipettor and spreading the inoculum to within approximately 3 mm of the edges of the carrier. The inoculated carriers were dried for 35 minutes at 35-37°C and 40% relative humidity with the Petri dish lids slightly ajar. For each lot of test substance, 5 test carriers per organism were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to expose for 10 seconds at room temperature (21.6-21.9°C) and at 16.9-18.3% relative humidity. Following the exposure period, the individual carriers with the excess liquid in each Petri dish were transferred at identical staggered intervals to 20 mL of D/E Neutralizing Broth. Within 30 minutes of neutralization, duplicate 1.00 ml and 0.100 ml aliquots of the neutralized solution (10°) were plated onto the Nutrient Agar recovery agar plate medium. The *S. aureus* plates were incubated

at 35-37°C for 48 hours. The *E. aerogenes* plates were incubated for 48 hours at 25-30°C. Following incubation, the subcultures were visually enumerated. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Protocol amendments reported in this study were reviewed.

27. MRID 493877-27, "Fungicidal Germicidal Spray Method," Test Organism: *Candida parapsilosis* (ATCC 22019) for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112, by Matthew Sathe, B.S. Study conducted at ATS Labs. Study completion date – Feb. 25, 2014. Project Number A16023.

This study was conducted against *Candida parapsilosis* (ATCC 22019). Testing was conducted for product Formula #e0029-002I, Batch 2001-106 and Batch 2001-112. These were tested using ATS Laboratory Protocol No. SRC62121113.FGS (copy provided). The product was received ready to use (RTU) aerosol spray. From stock, sufficient Sabouraud Dextrose Agar plates were inoculated with the test organism and were incubated for 2 days at 25-30°C. Following incubation, the organism was suspended in Butterfield's Buffer to appear heavier than a 4.0 McFarland turbidity standard. The test culture was mixed thoroughly prior to use. A 0.10 mL aliquot of FBS was added to 1.90 mL of prepared culture to yield a 5% fetal bovine serum organic soil load. 10 individual sterile glass slide carriers (18 mm x 36 mm) per batch, each in a Petri dish matted with two pieces of filter paper, were inoculated with 10.0 µL of test organism using a calibrated pipettor. Inoculum was uniformly spread over the test surface (approximately 1 square inch) of the slide in each Petri dish and covered immediately. This procedure was repeated until all slides were individually inoculated. The slides were allowed to dry for 30 minutes at 25-30°C and at 60% relative humidity. Carriers were used within 2 hours of drying. For each lot of test substance, test carriers were sprayed in a horizontal position with the test substance at a distance of 6-8 inches from the carrier surface for 2 seconds. The carriers were allowed to remain wet for 2 minutes at room temperature (20.0°C) and at 12.2% relative humidity. Following the exposure period, excess liquid was drained off the carrier and the individual carriers were transferred using sterile forceps at identical staggered intervals to 20 mL of primary Sabouraud Dextrose Broth + 0.14% Lecithin + 1.0% Tween 80 to neutralize. The carriers were then transferred to individual secondary subcultures containing 20 mL of the same subculture medium within approximately 25-60 minutes of the initial transfer. All subcultures were incubated for 3 days at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Carriers Tested		Carrier Population (Log ₁₀ CFU/ Carrier)
		Batch 2001-106	Batch 2001-112	
2 Minute Exposure Time				
493877-01	<i>Mycobacterium bovis</i> - BCG	0/10	0/10	4.69
493877-05	<i>Shigella flexneri</i> serotype 1B (ATCC 9380)	0/10	0/10	6.09

493877-06	<i>Salmonella enteritidis</i> (ATCC 4931)	0/10	0/10	5.85
493877-07	<i>Staphylococcus haemolyticus</i> (ATCC 29969)	0/10	0/10	5.27
493877-08	<i>Staphylococcus hominis</i> (ATCC 25615)	0/10	0/10	4.57
493877-09	<i>Staphylococcus saprophyticus</i> (ATCC 15305)	0/10	0/10	5.97
493877-10	<i>Micrococcus luteus</i> (ATCC 14408)	0/10*	0/10	3/27/14 – 6.12** 4/29/14 – 5.92***
493877-11	<i>Citrobacter freundii</i> (ATCC 8090)	0/10	0/10	5.94
493877-12	<i>Corynebacterium xerosis</i> (ATCC 7711)	1° = 0/10 2° = 0/10	1° = 0/10 2° = 0/10	4.86
493877-13	<i>Enterobacter cloacae</i> (ATCC 13047)	0/10	0/10	6.19
493877-14	<i>Klebsiella oxytoca</i> (ATCC 13182)	0/10	0/10	5.36
493877-15	<i>Shigella sonnei</i> (ATCC 25931)	0/10	0/10	5.27
493877-16	<i>Streptococcus agalactiae</i> (ATCC 13813)	1° = 0/10 2° = 0/10	1° = 0/10 2° = 0/10	5.06
493877-17	<i>Vibrio cholerae</i> (ATCC 11623)	0/10	0/10	4.05
493877-19	<i>Escherichia coli</i> O104:H21 (ATCC BAA-182)	0/10	0/10	5.58
493877-20	<i>Escherichia coli</i> 045: K-H- (ECL1001)	0/10	0/10	4.18
493877-21	<i>Escherichia coli</i> 0111:H8 (ATCC BAA-184)	0/10	0/10	5.42
493877-22	<i>Yersinia enterocolitica</i> (ATCC 23715)	0/10	0/10	4.86
493877-23	<i>Acinetobacter haemolyticus</i> (ATCC 17906)	0/10	0/10	5.72
493877-24	<i>Pasteurella multocida</i> (ATCC 12947)	0/10	0/10	6.45
493877-27	<i>Candida parapsilosis</i> (ATCC 22019)	1° = 0/10 2° = 0/10	1° = 0/10 2° = 0/10	5.06

*Without soil. Test with soil failed (1/10). **Test with soil. ***Test without soil.

MRID Number	Organism	No. Exhibiting Growth/ Total No. Carriers Tested	Carrier Population
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		Batch 2001-106	Batch 2001-112	(Log ₁₀ CFU/ Carrier)
3 Minute Exposure Time				
493877-02	<i>Mycobacterium bovis</i> - BCG	0/10	0/10	4.76
493877-18	<i>Bordetella avium</i> (ATCC 35086)	0/10	0/10	4.39

MRID Number	Organism	No. Exhibiting Growth/ Total No. Carriers Tested		Carrier Population (Log ₁₀ CFU/ Carrier)
		Batch 2001-106	Batch 2001-112	
5 Minute Exposure Time				
493877-03	<i>Mycobacterium bovis</i> - BCG	0/10	0/10	4.99

MRID Number	Organism	No. Exhibiting Growth/ Total No. Carriers Tested		Carrier Population (Log ₁₀ CFU/ Carrier)
		Batch 2001-106	Batch 2001-112	
6 Minute Exposure Time				
493877-04	<i>Mycobacterium bovis</i> - BCG	0/10	0/10	5.22

MRID Number	Organism	Dilution	Batch #2028-029	Batch #2028-030	Dried Virus Count
2 Minute Exposure Time					
493877-25	Avian Influenza A (H7N9) Virus, CDC # 013759189, Strain wildtype A/Anhui/1 /2013	10 ⁻¹ to 10 ⁻⁸	Complete Inactivation	Complete Inactivation	10 ^{5.75} TCID ₅₀ /0.1mL
		TCID ₅₀ /0.1mL	≤10 ^{0.50}	≤10 ^{0.50}	
		Log Reduction	>5.25	>5.25	

MRID Number	Organism	Lot No.	CFU/Carrier Average Log ₁₀	Percent Reduction	Carrier Population (Log ₁₀ CFU/Carrier)
10 Second Exposure Time					
493877-26	<i>Staphylococcus aureus</i> (ATCC 6538)	2001-106	<1.99	>99.9%	2/17/14=6.26
		2001-112	<1.46	>99.9%	1/20/14=6.49
493877-26	<i>Enterobacter aerogenes</i> (ATCC 13048)	2001-106	<2.25	>99.9%	2/17/14=6.96
		2001-112	<1.62	>99.9%	1/20/14=6.61

VI. CONCLUSION

1.) The submitted efficacy data **support** the ready-to-use product as a disinfectant against the following bacteria on hard, non-porous non- food contact surfaces with a 5% organic soil load for a 2-minute contact time at room temperature (20-23°C):

<i>Shigella flexneri</i> serotype 1B (ATCC 9380)	493877-05
<i>Salmonella enteritidis</i> (ATCC 4931)	493877-06
<i>Staphylococcus haemolyticus</i> (ATCC 29969)	493877-07
<i>Staphylococcus hominis</i> (ATCC 25615)	493877-08
<i>Staphylococcus saprophyticus</i> (ATCC 15305)	493877-09
<i>Citrobacter freundii</i> (ATCC 8090)	493877-11
<i>Corynebacterium xerosis</i> (ATCC 7711)	493877-12
<i>Enterobacter cloacae</i> (ATCC 13047)	493877-13
<i>Klebsiella oxytoca</i> (ATCC 13182)	493877-14
<i>Shigella sonnei</i> (ATCC 25931)	493877-15
<i>Streptococcus agalactiae</i> (ATCC 13813)	493877-16
<i>Vibrio cholerae</i> (ATCC 11623)	493877-17
<i>Escherichia coli</i> O104:H21 (ATCC BAA-182)	493877-19
<i>Escherichia coli</i> 045:K-H- (ECL1001)	493877-20
<i>Escherichia coli</i> 0111:H8 (ATCC BAA-184)	493877-21
<i>Yersinia enterocolitica</i> (ATCC 23715)	493877-22
<i>Acinetobacter haemolyticus</i> (ATCC 17906)	493877-23
<i>Pasteurella multocida</i> (ATCC 12947)	493877-24
<i>Neisseria elongata</i> (ATCC 25295)	492486-19

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

2.) The submitted efficacy data **support** the ready-to-use product as a disinfectant against the following bacterium **on pre-cleaned**, hard, non-porous non-food contact surfaces for contact times of 2 minutes, at room temperature:

<i>Micrococcus luteus</i> (ATCC 14408)	493877-10
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Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

3.) The submitted efficacy data **support** the ready-to-use product as a disinfectant against the following bacteria on hard, non-porous non- food contact surfaces with a 5% organic soil load for a 3-minute contact time at room temperature (21°C):

<i>Bordetella avium</i> (ATCC 35086)	493877-18
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Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth

4.) The submitted efficacy data **support** the ready-to-use product as a disinfectant against the following bacterium **on pre-cleaned** ,hard, non-porous non- food contact surfaces for contact times of 2, 3, and 5 minutes respectively, at room temperature (23-24°C):

<i>Mycobacterium bovis</i> – BCG	493877-01
<i>Mycobacterium bovis</i> – BCG	493877-02
<i>Mycobacterium bovis</i> – BCG	493877-03

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth

5.) The submitted efficacy data **support** the ready-to-use product as a disinfectant against the following bacterium on hard, non-porous non- food contact surfaces with a 5% organic soil load for a 6-minute contact time at room temperature (~24°C):

<i>Mycobacterium bovis</i> – BCG	493877-04
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Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth

6.) The submitted efficacy data **support** the ready-to-use product as a disinfectant against the following virus on hard, non-porous non- food contact surfaces with a 5% organic soil load for a 2-minute contact time at room temperature (20-21°C).

Avian Influenza A (H7N9) Virus, CDC # 013759189, Strain wildtype A/Anhui/1 /2013	493877-25
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Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Recoverable virus titers of at least 10⁴ were achieved. Complete inactivation (no growth) was indicated in all dilutions tested.

7.) The submitted efficacy data **support** the ready-to-use product) as a disinfectant against the following fungus on hard, non-porous non- food contact surfaces with a 5% organic soil load for a 2-minute contact time at room temperature (20°C):

<i>Candida parapsilosis</i> (ATCC 22019)	493877-27
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Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth

8.) The submitted efficacy data **support** the use of the ready-to-use product as a sanitizer against the following bacteria on hard non-porous non-food contact surfaces in the presence of 5% organic soil load for a 10-second contact time at room temperature (~21°C):

Staphylococcus aureus (ATCC 6538)
Enterobacter aerogenes (ATCC 13048)

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Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth. Neutralization confirmation testing showed positive growth of the microorganisms.

VI. LABEL

1. For label language (page 10) on Middle East Respiratory Syndrome (MERS), **remove "It and is expected to inactivate MERS-CoV when"** and read **"Used in accordance with the directions for use for Polio virus"**.

2. Efficacy against *Micrococcus luteus* (ATCC 14408) for contact time of 2 minutes and *Mycobacterium bovis* – BCG for contact times of 2, 3, and 5 minutes **must be on pre-cleaned** hard non-porous non-food contact surfaces. **Use directions must include pre-cleaning instructions.** Table on pages 14 and 15 must have **"Pre-cleaned surfaces"** instead of **"No soil"**

For "one-step" claims (effectiveness in the presence of 5% organic soil) use the term **"visibly clean surfaces"**.

3. The label claims that the ready-to-use product is a disinfectant against the following bacteria on hard, non-porous non-food contact surfaces for a 2 minute contact time (no mention is made of a soil organic load):

Neisseria elongata (ATCC 25295)
Shigella flexneri serotype 1B (ATCC 9380)
Salmonella enteritidis (ATCC 4931)
Staphylococcus haemolyticus (ATCC 29969)
Staphylococcus hominis (ATCC 25615)
Staphylococcus saprophyticus (ATCC 15305)
***Micrococcus luteus* (ATCC 14408)**
Citrobacter freundii (ATCC 8090)
Corynebacterium xerosis (ATCC 7711)
Enterobacter cloacae (ATCC 13047)
Klebsiella oxytoca (ATCC 13182)
Shigella sonnei (ATCC 25931)
Streptococcus agalactiae (ATCC 13813)
Vibrio cholerae (ATCC 11623)
Escherichia coli O104:H21 (ATCC BAA-182)
Escherichia coli 045 K-H- (ECL1001)
Escherichia coli 0111:H8 (ATCC BAA-184)
Yersinia enterocolitica (ATCC 23715)
Acinetobacter haemolyticus (ATCC 17906)
Pasteurella multocida (ATCC 12947)

These claims are acceptable as they are supported by the submitted data.

4. The label claims the ready-to-use product is a disinfectant *Mycobacterium bovis* – BCG, on hard non-porous non- food contact surfaces for a 6 minute contact time (no mention is made of a soil organic load).

This claim is acceptable as it is supported by the submitted data.

5. The label claims that the ready-to-use product is a disinfectant against Avian Influenza A (H7N9) Virus, CDC # 013759189, Strain wildtype A/Anhui/1 /2013, on hard non-porous non-food contact surfaces for a 2 minute contact time (no mention is made of a soil organic load).

This claim is acceptable as it is supported by the submitted data.

6. The label (p.8 and 13) claims that the ready-to-use product is a disinfectant against *Candida parapsilosis* (ATCC 22019), on hard non-porous non-food contact surfaces for a 2 minute contact time (no mention is made of a soil organic load).

This claim is acceptable as it is supported by the submitted data.

7. The label claims that the ready-to-use product is a sanitizer against the following bacteria on hard, non-porous, non-food contact/ soft surfaces for a 10 second contact time (no mention is made of a soil organic load):

Staphylococcus aureus (ATCC 6538)
Enterobacter aerogenes (ATCC 13048)

The claim for use on hard, non-porous, non-food contact surfaces is acceptable as it is supported by the submitted data. The claim for use on soft surfaces is not supported by submitted data.

8. On pages 8 and 9 of the proposed, remove "10 seconds" from soft surfaces claims and "disinfected" from sanitization claims.

9. On page 9 of the proposed label, under COLD / FLU, change "Helps stop the spread of Cold & Flu Viruses" to read "Helps stop the spread of Cold & Flu Viruses on treated environment surfaces."

10. On page 12 of the proposed, remove "No precleaning required", and add the terms "on treated environment surfaces" to "cross-contamination of germs" and "spread of germs".

11. On pages 17 and 18 of the proposed, under Use Direction, revise the term "Let stand for" to read "Surfaces must remain wet for".